LP4), demonstrated killing of C4-2 cells with IC_{50} values of 0.022 nM and 0.063 nM, and both showed maximum percent killing of 99%. Free reference payload MMAE demonstrated killing of C4-2 cells with IC_{50} value of 0.22 nM, and maximum percent killing of 99%. The unconjugated anti-STEAP2 antibody did not demonstrate any killing of C4-2 cells.

TABLE 14

Cytotoxicity in C4-2 cells by Tubulysin payloads, reference compounds, and ADCs

Test Articles	IC_{50} (nM)	% kill
Anti-STEAP2-LP3	0.097	99
Anti-STEAP2-LP4	0.15	99
Anti-STEAP2-MMAE	0.53	99
Isotype control-LP3	>50 nM	48
Isotype control-LP4	>50 nM	34
Isotype control-MMAE	>50 nM	16
Anti-STEAP2 Ab	>50 nM	0
IVd (payload of LP3)	0.022	99
Ve (payload of LP4)	0.063	99
MMAE (payload of LP8)	0.22	99

Anti-STEAP2 Antibodies

[0745] To determine the in vivo efficacy of anti-STEAP2 antibodies conjugated to tubulysins, studies were performed in immunocompromised mice bearing STEAP2 positive C_{4-2} prostate cancer xenografts.

[0746] For the assay, 7.5×10^6 C_{4-2} cells (ATCC, Cat # CRL-3314), which endogenously express STEAP2, were suspended in Matrigel (BD Biosciences, Cat #354234) and implanted subcutaneously into the left flank of male CB17 SCID mice (Taconic, Hudson N.Y.). Once tumors had reached an average volume of 220 mm^3 (around Day 15), mice were randomized into groups of 7 and given a single dose of either anti-STEAP2 conjugated antibodies (anti-STEAP2-LP3, anti-STEAP2-LP4, anti-STEAP2-MMAE), isotype control conjugated antibody, or vehicle at 2.5 mg/kg via tail vein injection. Tumors were measured with calipers twice a week until the average size of the vehicle group reached 1500 mm^3. Tumor size was calculated using the formula (length×width^2)/2 and the average tumor size+/−SEM was then calculated. Tumor growth inhibition was calculated according to the following formula: $(1-((T_{final}-T_{initial})/(C_{final}-C_{initial})))*100$, where treated group (T) and control group (C) represent the mean tumor mass on the day the vehicle group reached 1500 mm^3.

[0747] In this study, anti-STEAP2 antibody conjugated to MMAE was compared to anti-STEAP2 antibody conjugated to tubulysin linker-payloads (anti-STEAP2-LP3 and anti-STEAP2-LP4) for their ability to reduce C_{4-2} tumor size. As summarized in Table 15, treatment with anti-STEAP2-MMAE reference ADC resulted in an average of 81% tumor growth inhibition at the completion of the study. In comparison, treatment with anti-STEAP2-LP3 and Anti-STEAP2-LP4 ADCs demonstrated an average of 108% and 97% reduction in tumor growth, respectively. Treatment with the isotype control ADCs led to an average of 31-33% reduction in tumor growth. The anti-STEAP2 antibodies comprised N297Q mutations.

TABLE 15

Inhibition of C4-2 Tumor Growth at end of study in SCID mice treated with anti-STEAP2 ADCs

Treatment Group	Average Final Tumor size mm^3 (mean ± SEM)	Average Tumor Growth Inhibition (%)
PBS Vehicle	1539 ± 177	0
Isotype control-MMAE 2.5 mg/kg	1140 ± 213	31
Isotype control-LP3 2.5 mg/kg	1100 ± 202	33
Isotype control-LP4 2.5 mg/kg	1127 ± 192	31
Anti-STEAP2-MMAE 2.5 mg/kg	475 ± 118	81
Anti-STEAP2 N297Q-LP3 2.5 mg/kg	115 ± 6	108
Anti-STEAP2 N297Q-LP4 2.5 mg/kg	258 ± 60	97

Efficacy of STEAP2-Tubulysin ADC in CTG-2440 and CTG-2441 PDX Prostate Cancer Models

mAb Clone IDs:

[0748]

AbPID/REGN#	Common Name	N297Q mutation?
Isotype Control-LP4	Control Tubulysin ADC	Yes
Anti-STEAP2 N297Q Ab-LP4	STEAP2 Tubulysin ADC	Yes

Experimental Procedure:

[0749] Prostate cancer Patient-Derived Xenograft (PDX) tumor fragments of either CTG-2440 or CTG-2441 were implanted subcutaneously into the flank of male NOG mice. Once the tumor volumes reached approximately 200 mm^3, mice were randomized into groups of eight and were treated according to the schedule shown in Table 16 below. Tumor growth was monitored for 60 days post-implantation.